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1. The Occurrence and Distribution of the Larvae of Trombicula Akamushi Var. Deliensis as Related to the Epidemiology of Tsutsugamushi

/Following is a translation of an article written by Hsu Ping-k'uen, Su K'e-ch'in and Ch'en Hsin-t'ao of the Department of Parasitology, Chung-shan Medical College, appearing in Wei-sheng-wu Hsueh-pao (ACTA Microbiologica Sinica), Vol. 7, No. 1-2, Aug 1959, pp. 1-9./

The prevalence of tsutsugamushi disease in certain localities involves many unexplained factors. There are many ways to obtain a good comprehension of this problem, one being to study the relation between vector animals carrying infection and prevalence of the disease. Scientists have done a great deal to determine the vector animals carrying this infection. In this country /China/ vectors have been established to be Trombicula akamushi var. deliensis in Canton, and Trombicula akamushi in Formosa, while in other areas in the world such as Sumatra, India, Ceylon, Java, Phillipines, the Solomon Islands, and Australia, the vectors have been established to be Trombicula akamushi var. deliensis. And in some other areas as Japan and the Philippine Islands, Trombicula akamushi is found to be the vector. In the Malay Peninsula, Burma, and New Guinea, however, both Trombicula akamushi var. deliensis and Trombicula akamushi are found to be vectors. Judging from reports presented in literature from this nation and abroad, Trombicula akamushi var. deliensis and Trombicula akamushi are undoubted confirmed vectors responsible for tsutsugamushi in every area in the world and are the important ones.

During the past three years we have collected 43,793 larvae of Trombicula of various kinds from a number of animals available in Canton. Among those larvae 15,721 were those of Trombicula akamushi var. deliensis, while no larvae of Trombicula akamushi were found in the collection. Since Trombicula akamushi var. deliensis had been repeatedly proved to be vectors responsible for tsutsugamushi, this kind of mites had priority to be considered and studied. Later the authors completed surveys of distribution of localities were Trombicula akamushi var. deliensis were found. They also have conducted experiments on the relationship between the seasonal variance of occurrence as well as distribution of larvae of Trombicula akamushi var. deliensis on the one hand, and the climate elements on the other. Based on data from

the survey, and the experiments by the authors, and an analysis of regional situations in Canton plagued by tsutsugamushi disease, this report tries to present an explanation of seasonal and regional characteristics of the epidemiology of tsutsugamushi in Canton.

I. Monthly Variance of the Occurrence of the Larvae of Trombicula akamushi var. deliensis.

1. Variance during laboratory and incubation.

In our laboratory Trombicula akamushi var. deliensis was incubated in sealed tubes containing carbon powder; the constancy of saturated humidity in the tubes was maintained. To determine the seasonal characteristics of the occurrence of larvae of Trombicula akamushi var. deliensis, the larvae born by the mites in all tubes were collected once every 2 to 3 days and the total number of larvae collected was recorded. Consequently, the average number of larvae born by each mite (sexes of mites were disregarded) in every month was calculated and recorded. It must be made clear here that sexes of mites were very hard to determine while they were living and that there was more than one mite in each tube; it follows that the average number of larvae born by each female mite can not be computed. Nevertheless, these data still illustrate the seasonal variance of the occurrence of the larvae of Trombicula akamushi var. deliensis. Those recorded data (tabulated in Table 1 and illustrated in Fig. 5) indicate that the larvae incubated in the laboratory were collected in every month in the year except February and March. Studying curves showing monthly variance of the occurrence of the incubated larvae, we notice two peaks forming in regions of June-July and September-October, respectively, while a dip appears in the region of August. A comparison study of the two monthly variance curves showing the number of incubated larvae and average temperatures respectively gives us an idea about the dependence of the incubation on the average temperatures. (Variance of amount of rainfall and relative humidity may be ignored, since the incubation was conducted in sealed, carbon-powdered tubes in which constancy of a saturated humidity maintained in laboratory.) When the year is considered as a whole, December-March is rather a cold season with low average temperature for the city of Canton. During the period between December and March, January and February are the coldest months with average temperature ranging from 13.4 to 15.6° C. A few of larvae were collected as a result from incubation during

these two months while almost no larvae were collected during February and March after surviving the coldest months of January and February. The temperature is higher during April-November with average above 19° C., and more larvae were collected during this period. The temperature from May to October is even higher, with average above 24° C., and correspondingly more larvae were collected in this season. These experimental results have well illustrated a correlation between the number of incubated larvae and the average temperatures.

With the aid of data on incubation of larvae of Trombicula akamushi var. deliensis, we can also offer a good explanation for the occurrence of two peaks of June-July and September-October in each year. From these data, we found that for most Trombicula akamushi var. deliensis the culture, in which larvae become mites, has to survive one to four months with average temperature above 22° C.; that the culture, in which larvae become mites and mites give birth to larvae of the next generation, has to survive two to five months with average temperature above 22° C. Meteorological records for Canton indicate that April through October are the months with average temperature above 22° C., and that November through March are the months with average temperature below 20° C. (see Table 3). Therefore, in Canton usually those larvae coming into existence after September or October in one year and before May in the next year will give birth to larvae of the next generation in the June or July after they have grown up to be mites, while those larvae coming into existence in any of the months of June, July, and August, will give birth to larvae of the next generation in the following September of October. In other words, it is possible to incubate two generations within one year in Canton.

Incidentally, climatic elements other than temperature would also have something to do with the occurrence of the larvae of Trombicula akamushi var. deliensis. However, when saturated humidity is maintained (as mentioned above, incubations were conducted in carbon-powdered tubes in which saturated humidity was maintained), temperature would be the main climatic element which affects the occurrence of the larvae to a significant extent.

2. Monthly variance of the number of larvae of Trombicula akamushi var. deliensis carried by two kinds of common rodents.

Since 1954 we have examined several kinds of rodents, domestic pets, poultry, and wild birds and animals, commonly seen in the Canton area. Results of examations on those animals indicated that almost all of the larvae of Trombicula

akamushi var. deliensis in Canton were carried by four kinds of common rodents. They were very seldom carried by any other animals.

In the recent three years (March, 1954-October, 1957) we have captured 2,402 live rodents including 1,710 of Rattus novegicus, 453 of Rattus rattus, 159 of Rattus flavipectus, and 80 of Suncus murinus, all in the Canton metropolitan area. These 2,402 rodents were examined and seven kinds of larvae of Trombicula akamushi carried by them were collected. Out of those seven kinds, two were most commonly seen which included the larvae of Trombicula akamushi var. deliensis numbering 15,721. Since the four kinds of rodents mentioned above existed under different living conditions, we selected two of them which had the most frequent contact with residents in the city of Canton, which numbered more than 90% of the total number of rodents (2,163) captured in the recent three years, and which were the most common rodents in residential areas in Canton. These two kinds of rodents are Rattus novegicus and Rattus rattus. The results of examining these two kinds of rodents in each month were tabulated in Table II.

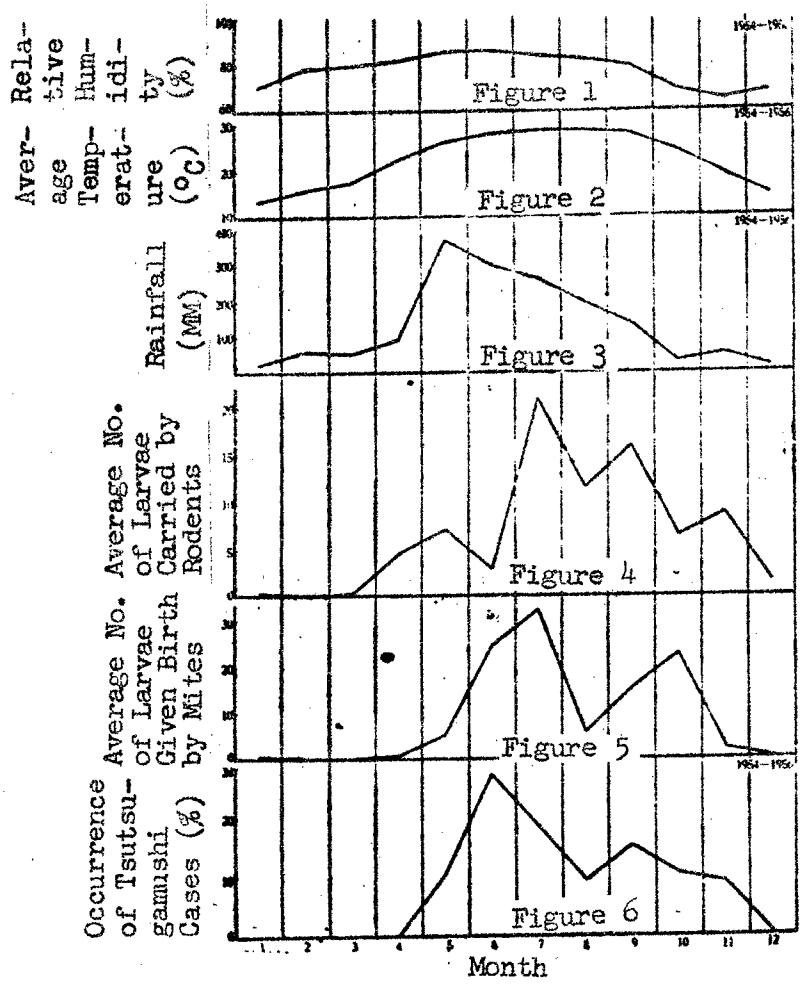
Figures in Table II and the curve in Fig. 4 indicated the seasonal variance of the larvae of Trombicula akamushi var. deliensis carried by two kinds of most common rodents. These experimental results are compared with laboratory incubation results and with the curve in Fig. 5, and agreement as well as disagreement are noted. The agreement is that a very small number of the larvae are carried by rodents during December-March in each year, more are carried during August, and a large number of the larvae are carried during April-November in each year. The disagreement is that the largest number of the larvae is incubated during June and October each year in the laboratory, while a decreasing number of the larvae is carried by rodents during June and October. We believe that this disagreement is concerned with rainfall occurring monthly in every year. This point will be later elaborated.

3. Discussion.

The cultivation of the larvae of Trombicula akamushi var. deliensis in the laboratory when saturated humidity was maintained indicates that Trombicula akamushi can multiply two generations in each year. It is likely that the monthly variance of occurrence of the larvae is mainly determined by temperature situations in the Canton area. For instance, development of the mites is retarded and the number of eggs deposited as well as the larvae incubated are decreased during December-March with the average temperature below 18° C. While the average temperature is generally above

Table 1. Monthly Variance of the Occurrence of the Larvae of Trombicula akamushi van deliensis Incubated in Laboratory.

Month	1	2	3	4	5	6	7	8	9	10	11	12
No. of Trombicula Akamushi (A)	15	15	14	22	39	37	30	37	36	24	17	16
No. of Larvae Incubated (L)	3	0	0	21	194	907	971	208	545	547	39	6
L/A	0.20	0	0	0.95	4.97	24.51	32.33	5.62	15.13	22.79	2.29	0.37



20° C. during the remaining seven months (April-October), multiplication of two generations of the mites is possible and two peaks in the curve showing the monthly variance of the occurrence of the larvae incubated in the laboratory are recorded. A point needing clarification here is that the man-made conditions including temperature, humidity, etc., are not completely identical with the natural conditions since saturated humidity is always maintained in the laboratories. (There is not too much difference between temperatures recorded under the two different situations mentioned above.)

The monthly variance of the occurrence of the larvae of Trombicula akamushi var. deliensis under natural conditions can be estimated by the monthly

Table 2. Monthly Variance of the Number of Larvae of Trombicula akamushi var. deliensis Carried by Two Kinds of Common Rodents (March, 1954-Oct. 1957).

Month	No. of Rodents Examined	Rodents Carrying Larvae		No. of Larvae Collected	Average No. of Larvae Carried by Each Rodent
		No.	%		
1	102	6	5.9	13	0.13
2	137	0	0	0	0
3	236	7	2.9	31	0.13
4	345	92	26.7	1569	4.5
5	316	108	34.2	2290	7.2
6	172	37	21.5	458	2.7
7	125	51	40.8	2612	20.9
8	234	80	59 9	2698	11 5
9	155	60	38.7	2343	15.1
10	148	49	33.1	954	6.4
11	57	18	31.6	501	8.8
12	132	24	18.2	213	1.6

variance of the number of the larvae carried by rodents examined. Some research workers even believe that the variance of the number of larvae of a certain kind of mite may serve as the index for the occurrence of the larvae in a certain area. And the authors think that the variance of the number of the larvae of Trombicula akamushi var. deliensis carried by common rodents, in certain senses, may indicate variance of occurrence of the larvae in a certain area. The terms "in certain senses" refer to general conditions under which the judgment is made; if any elements other than the general ones are involved, the judgment will not be the same. The reasons for our belief that under general conditions variance of the number of the larvae carried by rodents may indicate variance of the occurrence of the larvae are: (1) Examinations of various animals in Canton suggested that most of the larvae of Trombicula akamushi var. deliensis in Canton are carried by rodents, especially those commonly seen, and very few are carried by other animals.

Table 3. Meteorological Records for Canton During 1954-1956

Month	Rainfall (MM) 1954	1955	1956	Average	Average (°C.) Temperature (1954--1956)	Relative (%) Temperature (1954--1956)
1	30.69	12.22	29.93	24.15	13.4	70.91
2	68.75	8.73	94.8	57.42	15.6	78.47
3	107.54	20.52	28.81	52.29	17.75	79.87
4	170.24	35.59	66.28	90.69	22.63	81.83
5	240.35	558.17	305.63	368.06	26.13	85.57
6	308.10	388.51	201.39	299.34	27.77	85.67
7	118.69	532.01	135.46	262.04	28.47	83.67
8	191.4	257.95	99.13	182.84	28.47	82.33
9	280.69	96.35	30.43	135.72	27.9	79.33
10	19.49	71.11	4.45	31.68	24.27	69.33
11	96.05	22.66	54.1	54.27	19.33	65
12	4.64	8.63	42.4	18.56	14.8	68.33

(2) Surveys indicate that the mites are in existence wherever rodents are active. (3) Under general conditions the larvae distribute themselves on the ground in certain area surrounding the point where they originate. This agrees with what was found by Audy, Hanison, Suzuki, and Yu En-su. (4) Laboratory experiments showed that it usually takes two to five days for the larvae to feed themselves sufficiently to leave their hosts. From the above points we may assume that in Canton the variance of number of the larvae of Trombicula akamushi var. deliensis carried by rodents can reflect the variance of occurrence of larvae in that area. In Burma and the Malay Paninsula Audy and Hanison conducted investigations which indicate that although the ground distribution of the said larvae is somewhat limited to that area in which the birthplace of the larvae is the center of the radius, yet rainfall can interfere with such distribution. Therefore, in rainy seasons the larvae will extend their activity outward to avoid large amounts of rainfall. On the other hand, in the original activity areas where rodents usually appear, the density of the larvae will decrease and the number of larvae carried by rodents also decrease during a certain period of time. Following this argument, the noticeable decrease of the number of larvae carried by rodents during June, indicated by

data in Table II and the curve in Fig. 4, may be attributed to the heavy rainfall during May and June (368.06 mm for May and 299.34 mm for June.); while the decrease to a lesser degree during October-may be attributed to the heavy rainfall (280.69 mm) accumulated in September of 1954 (See Table III). If this should be true, then the monthly variance of the occurrence of larvae of Trombicula akamushi var. deliensis in nature is in agreement with that of the same larvae incubated in the laboratory for which there are also two peaks in June-July and September-October, respectively, every year. This conclusion further confirms the effect of temperation on the occurrence of the said larvae (refer to Sect. 2) without excluding the possible effects of other meteorological elements.

II. Monthly Variance of the Occurrence of Tsutsugamushi Cases

The monthly distribution of 209 cases of tsutsugamushi disease occurring in Canton during three years (1954--1956), as recorded in Table IV and reflected in Fig. 6, indicates that each year the occurrence of tsutsugamushi cases starts from May, reaches a peak in June (27.2%), declines in August (9%), rises in September (15.2%) and declines again in November and December. So there is one more peak next to the highest one for June-July in each year. If the Fig. 6 curve is compared with the curve in Fig. 5 showing variance of the occurrence of the larvae of Trombicula akamushi var. deliensis, a general agreement is noted, thus suggesting that whenever a large number of the larvae are in existence, tsutsugamushi disease cases occurred in large number also.

Next, the meteorological records for Canton during 1954--1956 (Table III) indicate that, among rainfall, relative humidity, and average temperature as three meteorological elements, the first one, e. g., the rainfall, is most closely related to the occurrence of tsutsugamushi disease cases. This is confirmed by a comparison of the Fig. 3 curve with that in Fig. 6. June and July, during which tsutsugamushi disease cases occur in largest number, are the months with heavy rainfall, especially June which is right after May, the month with the heaviest rainfall. For contrast, the curve showing the variance of rainfall each year starts to rise sharply in April and reaches its peak in May (368.06 mm), while the curve showing the variance of the occurrence of tsutsugamushi disease cases starts to rise in May and reaches its peak in June (27.2%). This comparison seems to suggest the occurrence of a large number

Table 4. Monthly Variance of the Occurrence of Tsutsugamushi Cases in Canton for Recent Three Years (1954--1956)

Month	1	2	3	4	5	6	7	8	9	10	11	12	Total
No. of Cases	0	0	0	0	21	57	39	19	32	21	19	1	209
%	0	0	0	0	10	27.2	18.6	9	15.2	10.5	9	0.5	100

of tsutsugamushi disease cases following a season with the heaviest rain. Soman and Das Menon made studies on 27 tsutsugamushi cases in Honei and they also observed that most of the cases occurred in September-October (or November) which immediately follow a rainy season. Workers in quarantine stations in Canton have the same experience that a certain number of tsutsukamushi cases always occur following consecutive rainy days. Rain itself, of course, can not cause the disease, nor has it any effect on the occurrence of the cases. It is the ground distribution of the larvae of Trombicula akamushi var. deliensis that is affected by rains. Investigations on areas where Trombicula var. deliensis exist in large number proved that there is a trace of the mites in residential areas with large population, but areas with sporadic inhabitants are swarming with mites. Therefore, the rainfall functions to enable the mites to have more chances of contacting people, and the possibility of spreading tsutsugamushi as a disease by these mites as vectors is correspondingly increased. Thus, it may be concluded that the reasons for the occurrence of large number of tsutsugamushi disease cases in Canton during June-July every year are: (1) The yearly occurrence of the largest number of the larvae of Trombicula akamushi var. deliensis is recorded in June-July. (2) June-July have a large amount of rainfall. (3) The month preceding June-July is the month with the heaviest rainfall. And the second peak shown in the curve indicating the occurrence of the larvae is responsible for the second peak in the curve during September-October showing the occurrence of cases.

III. Analysis of Physical Conditions in Areas Plagued by Tsutsugamushi

According to the occurrence of tsutsugamushi

caes, the plagued areas in Canton may be divided into two classes: (1) Sporadic areas: A few tsutsugamushi cases occur randomly in these areas. The general picture is as follows: one or two cases may occur in one place this year, and some other cases may occur there next year. A few cases occur in certain years, while none occur in other years. (2) Swarm areas: More cases occur with greater regularity in these areas every year. Swarm spots of Trombicula akamushi var. deliensis have been located in areas of both classes. But there has been more spots in areas of the second class than in areas of the first.

Studies on physical conditions in areas of both classes reveal distinctive differences between them.

Sporadic areas contain better buildings, roads, sanitary conditions, and drainage systems. There are no swampy lands or vacated and deserted lots with poor sanitary conditions. Vast vacated lots are not too frequent, and all of them are dry and clean. Pits or lots, with poor drainage systems are rare, so that even in rainy seasons there is no water-flooded area.

Contrasting conditions exist in swarm areas. Here swampy lands and vacated lots with poor drainage systems are noted. During rainy days they are flooded with water. These areas are fairly damp and the situation is not alleviated by some streams or brooks which have been flooded by water from neighboring hills during rainy seasons. Some of these regions are slum areas where physical establishments were repeatedly destroyed before the Communist seizure of regime and have yet to be reconstructed. Others of these areas have traditionally had poor buildings, roads, and sanitary conditions; and improvements after the Communist seizure of regime were not very extensive. In these areas the buildings are all one-story or wooden houses. There are vacated lots and deserted lands of vast area. The poor sanitary conditions cause the emergence of rodents in large number and close contact between men and rodents.

When we view the correlation between distribution of swarmed spots where Trombicula akamushi var. deliensis invade and activities of rodents, on the one hand, and that between the variance of amount of rainfall and the variance of distribution of the larvae of the same mites on the other, it is apparent that the physical conditions in these swarm areas favor the spread of tsutsugamushi disease by Trombicula akamushi var. deliensis both on rainy days and at other times. Laboratory incubation experiments indicate that humidity in the soil is a requirement for the development of the mites. The previous paragraphs stated that our investigations on swarm spots where the mites appear, suggest

that activities of rodents determine distribution of the mites. In some areas, environmental conditions favor development of the mites, and some slum sections favor activities of rodents. Thus it it is logical that swarm spots are more numerous where the mites exist, and that here the mites have more chance to contact people in these areas than in any other localities. This speculation has been supported by our own investigations on this point. Therefore, even during nice weather the mites in the said areas still have more chances to have contact with men than do those in other areas. In addition, wooden houses with simple structure can do nothing effective to fight the invasion by Trombicula larvae, nor the dispersion by the same larvae, caused by rains. And this dispersion is in turn supported by poor drainage systems to a certain extent. In short, damp slum areas favor multiplication of the mites and contact between the insects and men, while poor drainage systems promote dispersion of the larvae caused by rains. Eventually, these conditions favor the spread of tsutsugamushi disease in those areas.

IV. General Discussion

Investigations conducted by the authors in Canton during the recent three years indicate that spots swarming with Trombicula akamushi var. deliensis occur both in metropolitan areas with dense population and suburbs with fewer inhabitants. On the other hand, statistical data in this article show that larvae of Trombicula akamushi var. deliensis come into existence every month of each year except February. Since these mites have been proved to be vectors responsible for tsutsugamushi disease in the Canton area, the threat of invasion of tsutsugamushi as a disease through the mites can exist in every month of each year. But this threat varies in degree in different seasons and diverse areas. Concerning the seasons, they cause different chances to deliver rickettsial of tsutsugamushi by Trombicula larvae for the following reasons: (1) The occurrence of Trombicula larvae varies with seasons. For instance, this occurrence is in large number during May-October each year and reaches its peaks in June-July and September-October. During those periods, the changes for tsutsugamushi to be spread are far greater than during any other periods. (2) The ground distribution of the larvae is affected during certain periods -- May-June, for example, with its heavy rainfall. This effect generally increases changes of contact between the larvae and men, thus, in turn, promoting the chance of bacteria being delivered to infect men. The

combined effect of (1) and (2) increases the occurrence of tsutsugamushi cases. This may be why the cases occur in large number during June in the city of Canton. Concerning various areas, the number of distribution of spots swarming with Trombicula akamushi var. deliensis, the ground distribution of the larvae, and change of contact between the larvae and men are all determined by environmental conditions. In addition, these conditions may also affect the effect of rainfall on the ground distribution of the larvae. This may be the reason that tsutsugamushi cases very often are concentrated in certain areas in Canton. In summary, the seasonal variance of the occurrence of tsutsugamushi cases is caused by climatic conditions in one locality, while its local variance is caused by environmental conditions. However, since environmental factors need to be accounted for and these factors are changeable (partial changes, especially, are irregular), and since activities of men are not limited to certain areas, it may be believed that quite a number of cases may not be related to the factors mentioned above, and they are rather casual ones. Our study on date and locality of occurrence of a certain number of cases, with support from investigation on spots swarming with Trombicula akamushi var. deliensis, have endorsed this speculation to be factually true. The exclusion of casual cases decreases the number of cases to be analyzed; thus analysis was made on all the cases recorded in three years so that the quantity would be abundant.

Our ancestors noted the relationship between water and tsutsugamushi disease more than one thousand years ago. Ko Hung of the Chien Dynasty observed in his book called "Bau-pi-chi." "Lice exist both on land and in water. During rainy days they get on men while men are walking on sand." Another statement in a book called "Chow-hou-fan" declared: "There is sand every where -----the lice get on men while men are stepping in the water and walking through grass lands." The seasonal occurrence of tsutsugamushi and the effect of flood on this disease were also reported in Japanese literature many years ago. Tsutsugamushi was called "flood fever" just as it was called "river disease" by our ancestor Lee Shi--chen. About reasons behind existence of the relationship between water and tsutsugamushi, this article has offered points such as: the effect of rain or water on humidity of soil, requirement of humidity in soil by the mites, variance of amount of rainfall, and the effect of flood on ground distribution of the larvae, etc., to be preliminary explanations.

Statistical data in this article establish that certain climatic and environmental conditions are necessary for

the prevalence of tsutsugamushi disease. Therefore, improvement of environmental conditions is essential for diminishing the disease. The most important of all is improvement of sanitary situations. Swampy areas and deserted lots with poor sanitary conditions should not be allowed to continue in existence; houses and buildings should be altered to block invasion of rodents more effectively; and spots swarming with Trombicula akamushi should be thoroughly eliminated from inhabited areas. To improve environmental conditions the following should be done: mow the grass, clean the garbage, utilize vacated lands and deserted lots and fill up pits and swampy areas with earth or dirt containing mostly sand and very little rotten leaves. Chemicals like DDT and 666 may also be used, and drives to eliminating rodents may be initiated. Construction work to improve drainage systems and to prevent flood is also helpful. The measures suggested above have been proved to be effective judging from the results of the drive to eliminate tsutsugamushi disease obtained in recent years in the Canton area.

In addition to the measures suggested above, seasonal drives may also be initiated to fight the disease. The data on the monthly variance of occurrence of the larvae presented in this article suggest that the period between January and April -- the months preceding the occurrence of Trombicula larvae in large number -- is ideal season to begin the drive. Since during that period very few or none of Trombicula larvae are in existence, all the measures will surely be effective; on the other hand, also very few of the same larvae are carried by animals and since the few larvae in existence crowd at certain spots, they can easily and effectively eliminated. Also, the possibility of a wild burst of plague in May is diminished. Moreover, investigations on spots swarming with Trombicula akamushi var. deliensis indicate that if chemicals should be used to eliminate the mites on the ground, only parts of the areas instead of the whole areas need be treated with equally effective results. In so doing much work and insecticide may be saved for other uses.

V. Abstract

1. The results of study in the last 3 years, indicate that T. akamushi var. deliensis in the Canton region has a clear-cut seasonal distribution, the larvae being fewer in number from December to March, but in greatest quantity in June-July and September-October. Apparently the seasonal distribution is closely related to climatic conditions.

2. Cases of tsutsugamushi reported from Canton not only vary in seasonal distributions, but also in localities. In the last 3 years most of the cases have been reported in

June-July and September-October (coinciding with the two Trombicula peaks), particularly in June (27.2%). In some localities, the disease has been most prevalent in slum areas.

3. Meterological data indicate that cases of tsutsugamushi appear most abundantly after a month or so of heavy rain.

4. Temperature affects directly the number of Trombicula larvae that may appear at given times, and the heavy rain affects the dispersion of the larvae, hence increasing the change of contact between man and the vector. As a result, more cases of tsutsugamushi may eventually appear. Poor sanitary conditions in addition to favorable climatic conditions for the vectors aggravate the situation, so that an epidemic of tsutsugamushi may eventually break out. Further, environmental conditions may also alter one way or the other how rain affects the dispersal of the Trombicula larvae. These facts explain why cases are more concentrated in certain areas of the city.

5. On the basis of experiments and observations, methods of control of tsutsugamushi, including the choice of time, are proposed.

2. Studies on the Morphological Differences of Two Types of Trombicula Deliensis and the Transovarian Transmission of Rickettsia Orientalis

/Following is a translation of an article written by Yu En-shu and Wu Hsi-yi of Epidemic Diseases Research Institute, Fukien Province, appearing in Wei-sheng-wu Hsueh-pao (ACTA Microbiologica Sinica) Vol. 7, No. 1-2, May 1959, pp. 10-14./

The larvae of Trombicula deliensis collected from rats which we caught on Pin-t'ang Island, Fukien Province, were found to have two morphological types. In the previous article /1/ we reported on appearance, size, color, body distinctions of the larvae, their multiplying capability when cultivated in the laboratory, and their resistance to 666 and K_2S solution, respectively. Later the authors made some studies on baby and adult Trombicula deliensis and the inhibitory effect of some other drugs.

The authors also made studies on the life history of Trombicula carrying of Rickettsia orientalis by the vector during each of its transition stages, and the transovarian transmission of the bacteria. The following is a report on results from both of these groups of studies.

I. A further study on two morphological types of Trombicula deliensis

1) Morphology and distinctions of baby Trombicula and Trombicula deliensis.

On the basis of a study on Trombicula larvae, in the previous article, the larvae were divided into two groups, A and B. The larvae were cultivated to be baby Trombicula and Trombicula deliensis, and a sample was made from each. The samples of baby Trombicula were made before they had been fed with mosquito eggs, and those of Trombicula were made after the feeding. The measurements of baby Trombicula were tabulated in Tables I and II. It was found that the length and width of the body of a baby Trombicula in group A were greater than the counterparts of the baby mites in group B, as in the larvae; and the red color of the body of the baby mites in group A was deeper than that of the baby mites in group B. There was a noticeable difference between the lengths of foot and sensitive strip of group A baby mites and those of baby mites in group B; yet the distance between

sensitive points of the two groups of baby mites was almost the same.

Measuring 10 samples of baby Trombicula showed a prominent difference between lengths of the sensitive strip of baby Trombicula deliensis of the two groups. Then measurements of another 12 samples of baby Trombicula were taken. From the total 22 samples examined it was noticed that the length of the sensitive strip of group A baby Trombicula was longer than 96 um, the maximum length being 111 µm. Only two of the baby mites had shorter lengths of 85 µm and 88 µm, respectively. On the other hand, the lengths of the sensitive strip of group B baby mites were all shorter than 96 um, with none exceeding 98 µm. On the average, the length of the sensitive strip of group A baby mites was 102 µm and that of group B baby Trombicula was 88 µm.

Measuring the A and B groups of Trombicula deliensis samples indicated that group A mites were also larger than group B mites. An apparent difference was noticed in measuring the lengths of sensitive strips and feet. A similar difference was also observed in measuring distances between sensitive points of the two groups of mites. The difference was more marked than the counterpart noticed in measuring the larvae, but less noticeable than that observed in measuring baby mites. The average difference between distance from the 1st foot to the 4th foot of group A mites and that of group B mites was found to be 60, 55, 43, and 69 µm in 12 samples. The difference observed in measuring lengths of sensitive strips and distance between sensitive points were tabulated in table 3 and 4.

2) The different strength of resistance against dimethylphthalate possessed by larvae of Trombicula deliensis of groups A and B.

The different strength of resistance against 666 and K_2S solution possessed by the larvae of Trombula deliensis had been reported in the previous article. Here again dimethylphthalate was tested in the same fashion to determine its inhibitory effect on the larvae of Trombicula deliensis. In the test, dimethylphthalate was sprayed evenly at a piece of cloth by using an atomizer. Two or three days later, larvae of groups A and B that had been cultivated in the laboratory and had not been fed were placed on the piece of cloth. They were closely watched until they failed to move. The test was repeated 4 times using a total of 22 of group A larvae and 21 of group B. Each time, larvae of two groups were tested simultaneously for comparison. It was found that group A larvae failed to move after being exposed to the insecticide for a comparatively shorter period. A difference of 2-4 times of duration was recorded. Thus, group A larvae

Table 1. Measurements of Various Parts of The Body of Baby *Trombicula deliensis* of Groups A and B

Assigned No.	length of body	breast spread	back spread	length of the 1st foot	length of the 2nd foot	length of the 3rd foot	length of the 4th foot	length of sensitive strip	distance between sensitive points
group A 1	630	296	327	—	—	307	388	96	40
" 2	684	327	365	503	—	327	391	107	38
" 3	653	327	350	491	334	338	391	107	42
" 4	665	307	334	518	361	354	391	107	42
" 5	680	330	373	461	296	327	369	96	38
" 6	—	357	380	484	—	334	426	111	42
" 7	738	365	373	522	338	346	391	111	40
" 8	663	304	342	507	—	361	449	100	42
" 9	626	315	354	518	—	346	415	104	42
" 10	641	307	330	488	—	334	403	98	42
average	667	324	353	499	332	337	401	104	41
group B 1	633	288	311	449	307	315	373	94	40
" 2	680	296	311	461	304	—	—	92	40
" 3	638	292	342	441	261	296	342	84	42
" 4	653	304	342	468	273	300	369	86	42
" 5	645	284	330	392	261	296	338	86	44
" 6	603	265	307	438	261	284	334	84	38
" 7	553	261	296	438	281	277	334	90	40
" 8	595	277	315	453	273	273	361	86	40
" 9	622	282	307	422	269	281	323	84	40
" 10	630	288	311	441	288	296	331	84	38
average	625	284	317	440	278	299	345	87	40

Remark: Unit: μm

Table 2. Comparison of Length of Sensitive Strips of Baby *Trombicula deliensis* of Groups A and B

Length of Sensitive Strip (μm)	77	85	87	88	90	92	94	96	98	100	104	106	108	111
No. of Group A Baby Mites		1		1				4	1	2	4	1	4	4
No. of Group B Baby Mites	1	8	8	1	2	4	1	2						

Table 3. Comparison of Length of Sensitive Stips of *Trombicula deliensis* of Groups A and B

Lenth of Sensitive Stip (μm)	119	123	127	129	131	135	138	140	142	144	146	150	152	154	158	162	165	Total
No. of Group A Mites						3	1	1	6	3	4	6	1	4	3	3	1	36
No. of Group B Mites	2	1	4	1	4	11	2		3		7	1						36

Table 4. Comparison of Distance Between Sensitive Points of Trombicula deliensis of Groups A and B

Distance Between Sensitive Points (μm)	50	52	54	56	58	60	62	63	65	67	Total
No. of Group A Mites	2	2	8	1	16	4	3				36
No. of Group B Mites			2	2	9	3	9	4	6	1	36

Table 5. Time Elapsed Between Being Placed on Cloth Soaked with Dimethylphthalate of Larvae of Trombicula deliensis and Their Failure to Move

Run No.	Date	No. of Group A Larvae Tested	No. of Group B Larvae Tested	Difference in Time
I	1957,12,3	$\frac{20}{4}$, $\frac{30}{2}$, $\frac{20}{3}$ ($\frac{23}{3}$)	$\frac{15}{10}$ ($\frac{15}{10}$)	3.0
II	1958,1,6	$\frac{45}{1}$, $\frac{30}{1}$, $\frac{25}{1}$, $\frac{50}{1}$, $\frac{18}{1}$, $\frac{20}{1}$, $\frac{46}{1}$, $\frac{56}{1}$, $\frac{29}{1}$, $\frac{14}{3}$ ($\frac{51}{1}$)	$\frac{45}{6}$, $\frac{55}{10}$, $\frac{40}{5}$, $\frac{50}{5}$, $\frac{40}{6}$, $\frac{25}{7}$, $\frac{15}{7}$, $\frac{30}{9}$, $\frac{30}{9}$, $\frac{55}{3}$ ($\frac{21}{7}$)	4.0
III	1958,2,7	$\frac{4}{2}$, $\frac{50}{1}$, $\frac{35}{2}$, $\frac{41}{1}$, $\frac{15}{2}$, $\frac{59}{1}$ ($\frac{4}{2}$)	$\frac{30}{4}$, $\frac{35}{4}$, $\frac{30}{5}$, $\frac{20}{3}$, $\frac{55}{3}$, $\frac{0}{5}$ ($\frac{28}{4}$)	2.2
IV	1958,2,10	$\frac{10}{1}$, $\frac{15}{1}$, $\frac{55}{1}$, $\frac{55}{1}$, ($\frac{34}{1}$)	$\frac{20}{5}$, $\frac{20}{4}$, $\frac{40}{5}$, $\frac{15}{6}$ ($\frac{24}{5}$)	3.4

Fractions in Table V indicate time.
Denominators denote minutes; numerators denote seconds.
Average times are in parenthises.

had less strength of resistance to the insecticide than the smaller group B larvae.

II. The Transovarian Transmission of Rickettsia orientalis by Trombicula deliensis During Their Transition Stages

Trombicula deliensis of group A was chosen for experimentation since large numbers of these mites were available due to their speedy multiplication. Infected larvae carried by rodents were collected, cultivated in glass bottles, and developed to be baby and adult Trombiculae and larvae of the next generation. Samples of the insects in each transition stage were selected and a number of Rickettsia orientalis were isolated from them to study the transovarian transmission by Trombicula during each transition stage. The experimental procedures and results are here presented.

1) Source of Trombicula Larvae and Method of Cultivation.

In July, 1957 Rattus loseas were captured in the field. Any infected larvae found in earls of the rodents were collected and cultivated in glass tubes. The tubes had two open ends. One of the ends was sealed with a mixture of lime, carbon powder, and water. The other end was tightly stoppered by cotton balls to keep larvae inside the tubes. The tubes were placed in a vessel filled with enough water and the larvae were incubated at 23-30° C.

The baby and adult Trombiculae were fed with mosquito eggs once every one to two days. Larvae of the second generation, collected from incubated eggs of the mites were placed into the ears of rodents and fed by sucking blood of the rodents. After 43 hours the larvae were fully fed and were removed from the ears of the rodents and incubated in the same manner. Those Rattus loseas bitten by larvae had been caught from places plagued by Tsutsugamushi. It was hard to say whether the rodents were infected or not. But some of the larvae were placed in the ears of rodents which had been experimently infected by tsutsugamushi disease.

2) Isolation and Identification of Rickettsia orientalis.

During incubation periods, the insects in transition stages such as baby and adult Trombiculae and larvae of the next generation were selected for isolating of Rickettsia orientalis. Groups formed by 40-50 baby or adult Trombiculae and groups formed by more than 100 larvae were ground and mixed with 1-2 ml of saline. The mixtures were treated with antibiotics at 4° C. for three hours. One to two mice were injected intra-abdominally with this mixture. During cultiva-

tion, whenever deaths of mice occurred, the infection was transferred to other mice. Mice not infected after 21 days were also disposed of. In any case the mice were subsequently dissected and examined. If abdominal juice was available, slides were made and stained. If no infection was noticeable, then hepatic juice, spleen juice, and kidney juice of the mice were inoculated to other mice. If still no infection was observed, the result was determined as negative.

Isolated Rickettsiae orientalis were stored after genetic characteristics and their infectivity toward mice were observed. The bacteria were also treated with established Rickettsia orientalis as an immune test for their final identification.

3) Experimental Results.

A) Isolation of Rickettsia orientalis from young Trombiculae: The first experiment was carried out on 5 November 1957, in which abdominal juice of mice infected by Rickettsia orientalis was used to infect Rattus loseas. During 18-23 November in the same year Trombicula larvae which had been incubated in the laboratory were placed in the ears of Rattus loseas to be fed. After the larvae had been fully fed they were removed and continued being fed to become young Trombicula. About one month later, on 9 January 1958, 200 infected baby Trombiculae were divided into two groups and made into portions of a suspension. Each portion of suspension was injected into two mice. On 27 January two Rattus loseas of one group had some symptoms of being infected. The hair of the mice was standing up and their abdomens were swollen. They were inactive and exhausted. Dissection indicated lymphadenitis, skin hemorrhage, a small amount of abdominal secretion, swollen spleen, attachment of liver and stomach, and secretion in chest. Slides were prepared from the abdominal secretion and examined. Many Rickettsiae orientalis were found in the cells. A mixed suspension of abdominal secretion and secretion of spleen, liver, and kidney were prepared and placed on blood-agar and agar-agar slopes for antiseptic cultivation. Four other mice were also injected and nine days later showed the same symptoms. These Rickettsiae orientalis were treated with the same bacteria of known identity for immune test which proved their expected identification.

Two mice of another group were dissected at the same time and no infection was noted. Secretion of liver, spleen, and kidney of these mice was injected into other mice and negative results were again obtained. The second experiment was carried out in the middle of December, 1957, in which Rattus loseas were infected with Rickettsia samples. Nine days later Trombicula larvae were placed in ears of these

rats for feeding. There were then incubated to become young Trombiculae. After five days a suspension was prepared from 110 young Trombiculae and injected into mice; no infection was observed after three weeks. The same secretion was injected into mice of another generation, and negative results were again obtained.

A total of three groups of mice were used for experiment; and positive results were obtained on only one of these three.

B) Isolation of Rickettsia orientalis from Trombicula:

Six groups of Trombiculae were used. Employing two groups of Trombicula with 34 and 41 in each group, respectively, the larvae were fed into ears of Rattus loseas which had been infected. Rickettsia orientalis was isolated from the two-month-old mites with negative results.

Four other groups of Trombicula had 48,50,50,43, respectively, in each group. Larvae of the mites were fed into ears of Rattus loseas which had been caught in the field. Rickettsiae orientalis were isolated from the six-month-old mites with negative results, also.

C) Isolation of Rickettsia orientalis from larvae of second generation:

Fully fed Trombicula larvae collected from Rattus loseas which had been caught in the field were cultivated in glass bottles in the laboratory and developed to be young and adult Trombicula and larvae of the second generation was prepared from 160 of these larvae. A suspension before they were fed into ears of Rattus loseas. The suspension was injected into two mice. One of them had symptoms of infection. Dissection gave the same result. When slides were made, a large number of Rickettsia orientalis were found in the cells. Immune test with known Rickettsia orientalis confirmed the identification.

All apparatus for cultivation of Trombicula was carefully prevented from touching apparatus for Rickettsia orientalis samples. Cultivations were carried out in separate rooms with mosquito eggs as the only food. Therefore, it may be concluded that Rickettsia orientalis carried by larvae of the second generation was inherited from larvae of the previous generation. Thus, the transovarian transmission of the bacteria was eventually confirmed.

Discussion

The epidemiology of tsutsugamushi disease is rather complicated, and some of its problems are still to be solved. Such problems are exemplified by the epidemiological relationship between Trombicula akamushi as the vector and the rodent

as the host, and by the situation of Rickettsia orientalis being carried by Trombicula akamushi during each stage of development. These problems are closely related to clarification of the natural source of epidemicity of tsutsugamushi and development of epidemic prevention measures. However, no adequate studies on these problems have been carried out. The outstanding problem now is whether Trombicula larvae can be infected by the infected hosts. Some bacteriologists do not believe that this passage of infection is possible. But what would be the passage through which tsutsugamushi disease of long history has been inherited? How much does the transovarian transmission account for the inheritance? These are problems whose solutions are urgently needed.

The transovarian transmission of Rickettsia orientalis in the body of Trombicula was proved by research workers. Krishnan [4] and Audy [5], and others proved that in the Southwest Pacific area transovarian transmission of Rickettsia orientalis may be effective for two or three generations and larvae of the second generation can again infect healthy animals. However, not too much success in isolating Rickettsia orientalis from Trombicula akamushi in transition stages other than the larvae stage has been recorded. No report on successful isolation of this bacterium from baby Trombicula is available in literature either [2, 3], thus making proper understanding of transovarian transmission impossible. Nevertheless, in our own experiment Rickettsia orientalis were isolated from young Trombicula and larvae of next generation. This result indicates that the bacteria can be transmitted to Trombicula larvae of next generation, and that the bacteria can be isolated from baby Trombicula. Although no positive results were ever obtained from Trombicula, it still may be speculated that the bacteria can also be carried by Trombicula and in their eggs. The past failure of isolating the bacteria from baby Trombicula may be caused by improper selection of specimen and poor timing of laboratory operations. As for the hypothesis [3] that Rickettsia orientalis tends to be weaker during transition stages other than the larval stage, we are not able to prove it.

It was stated in the previous article that the two types of Trombicula var. deliensis have different strength of resistance against 666 and K_2S. In this experiment it was proved that the mites of two types also have different strength of resistance against dimethylphthalate. Strength of such resistance is not regular. For instance, against K_2S solution Trombicula larvae of group A have stronger resistance, while against 666 and dimethylphthalate Trombi-

cula larvae of group B have stronger resistance. This finding is rather significant. But the explanation for this is not available.

IV. Summary

1. Based on their morphological differences, Trombicula deliensis was divided into two groups, A and B. The differences between Trombicula larvae of two groups had been reported in the previous article, and those between baby Trombicula of two groups and between adult Trombicula of two groups were reported here in this article. Like their larvae, young and adult Trombiculae of group A have deeper red for their body color, greater size, longer feet, and longer sensitive strips.

2. Two types of larvae of Trombicula var. deliensis have different strength of resistance against dimethylphthalate. Group A larvae have weaker resistance than group B larvae, the difference ranging 2-4 times in strength which is inversely proportional to their sizes. This result is in line with that obtained from testing resistance of larvae against 666, while it is contradictory to testing resistance of larvae against K_2S solution.

3. Learning from results of the previous and the current experiments, we believe that it is advisable to divide Trombicula akamushu var. deliensis into two groups. And the finding that larvae of the two groups have different strength of resistance against insecticides is highly significant.

4. It was proved that Rickettsia orientalis can be delivered by group A Trombicula to larvae of the next generation through transovarian transmission. Isolation of Rickettsia orientalis from young Trombicula was proved to be successful for the first time. According to literature available and our experimental results, it is believed that Rickettsia orientalis is carried by Trombicula var. deliensis during each of its transition stages.

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3. Observation on the Reversion of Atypical Dysentery Bacilli to Typical Form

/Following is a translation of excerpts from an article written by Kung T'ien-en of the Clinical Department, #175 Hospital, Chinese Army. The article appeared in Wei-sheng-wu Hsueh-pao (ACTA Microbiologica Sinica), Vol. 7, No. 1-2, May 1959, pp. 103-107./

In 1957 we accepted most of new dysentery patients for clinical treatment, and we had the opportunity to isolate 149 different strains of dysentery bacilli. From the process of isolation and identification we found that 13 of those strains had biochemical activities entirely different from those of typical dysentery bacilli, yet when they were treated with dysentery serums of multiple valence, agglutination occurred on the slide. To determine the nature of these bacterial strains we collected them and made systemic studies and close observations.

The biological hypotheses originating from dialectic materialism advocated by Mi Ch'iu-lin and Lee Sun-k'o is the ideological guidance for our research work. The arguments suggest that the entire process of growth, the nature of heredity, and the process of reversion are determined by nutrition or sources of life. Therefore we must first assume that those bacterial strains are either atypical strains or mixed strains caused by nutritious allogamy. The reason for this assumption is that while dysentery bacilli are multiplying in human bodies their growing functions may be influenced by nutrition and circumstantial factors. However, the quantitative and qualitative changes of the influenced growing process have not been sufficiently consistent to be considered an established rule, since they are prominently demonstrated only during the fermentation process. It is possible that bacterial strains reverted to their original strains through "short" inheritance? Using this ideological point of view, the author carried out experiments in which the method to be introduced in this article was used to revert 13 atypical dysentery strains back to their original breeds. The experimental results justified our assumptions and the biological hypotheses of Mi Ch'iu Lin.

Specimens and Procedures

Source of dysentery bacilli strains: The 13 strains

tested in this experiment were isolated from the stool of hospitalized patients by our laboratory at Nan-ch'an, Kiang-si in 1957. The bacterial strains were so chosen that they demonstrated noticeable agglutination towards dysentery polyvalence serums or flexneri polyvalence serums on slides, while their biochemical activities were nontypical or different from those of standard bacilli.

Preparation of culture media: 20 gm of peptone, 5 gm of NaCl, 3 gm of Na_2PO_4, and 25 gm of agar were weighed out and placed in a one-liter flask which contained 1000 cc of distilled water. The mixture was heated to dissolve and its pH was adjusted to 7.4. It was then filtered through four layers of gauze. 10 gm of soluble starch and 5 gm of glucose were next placed in a small beaker and dissolved in a small amount of water. This mixture was added to the prepared agar media and mixed well. The final material was distributed into several test tubes and was sterilized with 8 lbs. pressure for 20 minutes. Slopes were prepared from the culture media, tested for contamination, and were then ready to be used.

Inheritable bacterial strains grew in this type of medium more prosperously and satisfactorily than they did in regular agar medium.

Method of inheritance: Suspected colony bacteria were scratched off from a plate of indigo and methylene blue with a platinum loop, inoculated onto media of disaccharides containing iron, and incubated at 37° C. for 24 hours. The bacteria were tested qualitatively by agglutination reaction on slides toward dysentery bacilli of polyvalence and results were recorded. Then colony bacteria were again scratched off media of disaccharides, inoculated into five carbohydrate tubes and other biochemical media, and were incubated at 37° C. for 24 hours; the results were recorded. Those carbohydrate tubes in which positive reaction was observed were incubated for one week. The above process was considered in this experiment as two generations; within these two generations no agglutination tests were done quantitatively.

These bacterial strains to be tested were inoculated on slope media of starch-agar mixture and incubated at 37° C. for 24 hours. The cultivated colony bacterial were scratched off, quantitatively agglutination-tested generation by generation, and transferred by inoculation into media; after repeated transplantations those bacterial strains whose agglutination reactions were slowed down by decreasing concentrations of dysentery serum of polyvalence were discarded, while those which showed agglutination reaction even toward serum of concentration 1 : 5120 (effective valence of serums 6400) were considered to be reverted strains which had been changed from

typical form to atypical form; at the same time, biochemical tests were also used on those strains to make final determination.

The test on anti-sulfonamide drugs using Moroz's method was followed to prepare culture media.

Clinical Specimens

A total of 12 of 13 atypical bacterial specimens tested in this experiment were collected from patients suffering from chronic dysentery; the other one was missed because of careless ness. Among those 12 specimens isolated, six were flexneri bacilli, one was sonnei and one was smith bacilli. Among the four other specimens, no dysentery bacilli were observed after repeated cultivation. Of 12 specimens, nine were infected with complex amebae and one had Enteromonas hominis in it. All the patients from whom the specimens were isolated were treated with amidine, yatren carbarsone, and sulfonamide; some of them were also treated with coptis root, syntomycin, or antitoxin, or was given an enema with scallion juce. Although all the cases had been repeatedly treated with several kinds of drugs, the patients still had bowel movements three to four times or more per day, and mucus was found in stool. During the later stages, however, no typical dysentery bacilli were found after the stool was repeatedly cultivated.

Characteristics of the Original Bacterial Strains Bacterial colony: After being inoculated on agar plate and incubated at 37° C. for 24 hours, the bacterial colony was smooth, colorless, and of spherical shape and serrated. The colonies scattered to small transparent or semi-transparent groups. Then the colony groups were scratched by platinum wire, there was no sticky tension between the wire and the bacterial groups. Motion: The bacilli were motionless or had very little motion. Stain: Gram's method of staining was used. The bacteria were Gram-positive and appeared as straight bacilli with round ends and of medium size. No cilium staining was done owing to shortage of laboratory workers. Biochemical reactions: Detail is tabulated in Table 1. No decomposition, decomposition without gas, or decomposition with gas evolution were three different biochemical reactions by the strains towards various carbohydrates.

All the bacterial strains grew evenly except one, they formed a thin layer and caused a small amount of precipitate. According to their biochemical activities, the 13 atypical dysentery strains may be divided into seven different types.

Reactions towards serums: 13 strains showed different degrees of agglutination towards dysentery serums of polyvalence with concentration of agglutination ranging from 1:4 to 1:640. As for various serums of monovalence, weak agglutination was noticed only in flexneri serums of polyvalence. These serums were prepared by Shanghai Biological Products Research Service.

Towards Salmonella anti-serum (A-E group) of polyvalence (products of Ta-lien Biological Products Research Service): Seven strains showed agglutination and six strains did not. No test of anti-serums of monovalence was run on those seven strains which had shown agglutination towards Salmonella serums of polyvalence, as a result of a shortage of laboratory workers.

Anti-sulfonamide drugs test: All 13 atypical strains had resistance to sulfaguanidine, sulfathiazole, and sulfadiazine. 11 of those 13 atypical strains also showed sensitivity to chloromycetin. Biochemical activities and agglutination reaction of the strains after inheritance are listed in Tables 1 and 2.

Discussion

Variation of bacterial strain was well recognized during the age of Pasteur. Yet the true nature of this variation had not been correctly determined owing to hindrance by the non-inheritance theory advocated by Weissmann and Morgan. After the socialistic October Revolution, the I-mi-ping-lin biological hypotheses were highly recognized and rapidly developed. Russian bacteriologists have since introduced so much new experimental evidence and information concerning theories on bacterial variation that bacteriology is enriched as a branch of the natural sciences; theories on bacterial variation have been corrected; and a sound foundation is laid for utilization and recognition of bacteria whose inherited characters are varied. The variation of dysentery bacilli may be caused by mutual reactions between a harmless bacterial group and dysentery bacilli in the intestine. Some bacteriologists believe that atypical dysentery bacilli are coli communis bacilli or coli communior bacilli which have acquired whole or part of the characteristics of dysentery bacilli by being influenced by dysentery bacilli or their intermediate metabolites. Some other bacteriologists, however, suggest that atypical dysentery bacilli are those dysentery bacilli which have lost part of the characteristics of typical dysentery bacilli and have acquired part of the characteristics of coli communis bacilli and coli communior bacilli by being influenced by the latter

two bacilli or by their metabolites. It seems that the latter postulate coincides with our experimental results. Nevertheless, we do not think that the two postulates mentioned above are contradictory to each other, since superiority in heredity of either one of the dysentery and coli bacilli while they are in coexistence makes the difference. It is apparent that dysentery bacilli are predominant factors in organisms of patients suffering from acute type of dysentery, that coli bacilli are predominant factors in those of patients suffering from a chronic type of the same disease, and that the mutual influences between dysentery and coli bacilli differ depending on application of either case. This hypothesis had been verified by V. B. M. Mesnyayeva and was proved in this experiment also. Mesnyayeva did experiments in which bacterial strains, which demonstrated the agglutination reaction towards certain kinds of dysentery serums and showed no typical biochemical activities, were isolated from stool of patients suffering chronic dysentery or being in recovery stages, and were cultivated in Bakto-K medium; after being cultivated in such medium the bacterial strains were reverted back to typical flexneri bacilli. The 12 strains, which had been collected from patients suffering from chronic dysentery and were tested in this experiment, were dysentery bacilli existing in the intestine for a long time, and they acquired certain new characteristics after being influenced by harmless bacterial groups in the intestine.

Summary

1. Thirteen atypical strains of dysentery bacilli were isolated from stool of patients suffering from chronic type of dysentery in 12 cases. (Another specimen was missed due to carelessness.) The bacterial strains are easily mistaken for coli communis or coli communior bacilli, since they have similar biochemical activities.

2. Antigenicities to varying extent were preserved in those 13 atypical strains which showed agglutination (40--640x) towards dysentery serums of polyvalence.

3. The 13 atypical strains recovered their typical biochemical characteristics after 5-17 generations of "short" inheritance and were able to have agglutination reaction toward certain serums of high dilution. As a result, 12 strains out of 13 were reverted to flexneri bacilli and the other one to sonnei bacillus.

4. The 13 atypical strains, both before and after their reversion, had resistance to sulfaguanidine, sulfathiozole, and sulfadiazine; two of them also showed resist-

ance to chloromycetin. The resistance of the strains to those drugs was neither increased nor decreased through the reversion process.

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4. Initial Report on the Application of the Hemagglutination Test in the Early and Rapid Diagnosis of Dysentery

/Following is a translation of an article written by Wu K'ai-yu and Cheng Kuo-k'uei of Epidemic Diseases Research Institute, Fukien Province, appearing in Wei-sheng-wu Hsueh-pao (ACTA Microbiologica Sinica), Vol. 7, No. 1-2, May 1959, pp. 88-92./

The most reliable basis for diagnosis of dysentery is believed to be the cultivation of bacteria which cause the disease, whether the disease is of acute or chronic type. Successful bacterial cultivation requires quite a long time and certain equipment and techniques. Bacteriaologists have been trying to shorten the time required for bacterial cultivation and to improve techniques so as to justify using the cultivation as a really reliable foundation for diagnosis. However, the positivity rate and the time fact in bacterial cultivation still pose problems for diagnosis of typical or special cases of dysentery as an epidemic disease. To make early and rapid diagnosis of dysentery possible, Russian workers recommend the rapid agglutination test and the test on a precipitate of semi-antigens, yet bacteriologists hold different views on characteristics of these tests. /1/ This confusion prompts the authors to devote their efforts to a method of early and prompt diagnosis of dysentery other than those methods mentioned above.

The method which the authors have been studying is called the hemagglutination test (hereinafter referred to as HA) that may be applied in diagnosing many diseases of epidemic nature. /2/ The application of this test in diagnosis of dysentery has never discussed in the literature. In 1956 Neter suggested the possibility of hemagglutination caused by coli bacilli and of application of such reaction in diagnosis. /3/ The main point of his report was the detection of specific antibodies /3/ in the human body or immune serums by hemagglutination. In the same year Suzuki Takeo reported the method of hemagglutination by "o" antigens of coli bacilli. /4/ Then it was learned from the authors' experiments that the sensitivity and selectivity of "o" antigens of dysentery bacilli to HA is no less acute than those to the bacterial agglutination reaction, and that the method for the former is easier than that for the latter. /6/ But whether erythrocytes infected by "o" antigens prepared

from boiling stool of patients suffering from the disease in the early stages can be used in HA test for early diagnosis is a problem to be solved. In 1957 the authors ran HA tests on 311 cases while they were doing bacterial tests on the stool of patients suffering from dysentery of acute type. They also selected 100 out of those 311 cases, ran rapid agglutination tests on them and compared positivity rates in experimental results which had been obtained from three different methods. The authors also tried to determine whether coli bacilli in stool of the healthy persons may interfere with the specificity of this method, and the findings are here reported.

Specimens and Procedures

1. Baterial examination: Bacterial specimens were collected from the rectum of patients three consecutive times at an interval of one or two days. The specimens were seeded directly on media of indigo and methylene blue. After being incubated for 18-24 hours, cultures were scratched and inoculated to media of ferro-disaccharides. The isolated pure cultures were inoculated again into fermentation tubes of various carbohydrates and observed for three weeks, and inoculated to semi-solid media as well. Tests for indole, H_2S, M.R., V.-P., utilization of sodium citrate and urea decomposition were made and test tube agglutination reaction resulted. (Diagnostic serum was prepared by Shanghai Biological Products Service.)

2. Rapid agglutination reaction: A Russian method was used.

3. Hemagglutination test.

(1) Preparation of antigens: While bacterial strains were being cultivated, stool specimens were stirred well, inoculated on regular agar slopes of pH 7.6, and incubated at 37° C. for 16-24 hours. The cultures were washed off by 3 ml of saline (or buffered NaCl solution of pH 7.6), heated to boiling for two hours, and centrifuged. The precipitate was washed three times with saline and finally a buffering NaCl solution of pH 6.4 was added. The material was shaken well and ready to be used. (Suspension in centrifuge tubes might also be collected as antigens.)

(2) The method for making diluted solution of erythrocytes and antiserums was described in another article.

(3) Infections of erythrocytes: 0.0375 ml of packed erythrocytes was added to 1.5 ml of antigens prepared in (1) to make a concentration of 2.5% of red cells. (Another tube was filled with saline for comparison.) The mixture was mixed well, incubated at 37° C. for two hours, and

shaken occasionally. It was then washed three times by Centrifuging at 500-1000 rpm with buffered NaCl solution of pH 6.4 and decanted. The same amount of diluted solution as that of the original solution was added to make a suspension of erythrocytes of concentration 2.5%.

(4) The actual hemagglutination test: A 0.05 ml portion of erythrocytes infected by "o" antigens which had been prepared from stool culture of patients was added to tubes in a row containing flexmeri and sonnei diagnostic serums and regular serum of different dilutions (starting from 1:160). The mixtures were shaken thoroughly and incubated at 37° C. for two hours.

(5) Evaluation of experimental results: Those which were of concentration exceeding 1:320 were considered positive.

Experimental Results

While stool culture of patients whose diagnosis was acute dysentery in 311 clinical cases were being cultivated, HA tests were also processed with results compared in Table 1.

Table 1. Comparison of Results from Bacterial Cultivation and HA Test

Method / No. of Cases	Bacterial Cultivation		HA Test	
	Patients with Positive Reaction	%	Patients with Positive Reaction	%
311	136	43.7	164	52.7

Table 1 indicates that the percentage of positivity rate found in HA tests was 9-10% higher than that found in bacterial cultivations. Of 311 cases, 74% showed that results from bacterial cultivation were in accord with HA test; 26% showed results from the two kinds of reactions not in accordance of one another. The results were tabulated in Table 2.

Table 2. Comparison of Results from Bacterial Cultivation and HA Test

Agreement				Disagreement			
Bacterial Cultivation	HA Test	Cases	%	Bacterial Cultivation	HA test	Cases	%
Negative	Neg.	120	38.7	Negative	Pos.	49	15.8
Positive	Pos.	106	34.0		1.fl.	24	
1.flexneri	1.fl.	77			2.so.	25	
2.sonnei	2.so.	29		Negative	Pos.	4	1.3
Positive	Pos.	4	1.3	Positive	Neg.	22	7.0
1.flexneri	1.fl.			1.flexneri		19	
2.sonnei	2.so.	2		2.sonnei		3	
				flexneri was cultivated.	HA:so.	5	1.6
				sonnei was cultivated.	HA:fl	1	
Total		230	74.0			81	26.0

Table 2 indicates that in 230 out of 311 cases (74%), the results from bacterial cultivations agreed with HA tests, and in 81 out of those 311 cases the results disagreed with each other. Among the results in disagreement more than one-half of the cases (15.8%) were those that were positive in bacterial cultivations and positive in HA tests. Those cases were all diagnosed as acute dysentery. On the other hand, 7.0% of the total cases were those that were positive in bacterial cultivation and negative in HA tests.

The sensitivity of erythrocytes infected by "o" antigens which had been prepared from boiling stool culture to hemagglutination was analyzed in 106 cases in which positive reactions were observed both in bacterial examinations and HA tests. The analysis is shown in Table 3.

Table 3 indicates that among 77 cases in which flexneri bacilli were cultivated and positive HA reaction on flexneri serum was noticed, 88.3% showed a concentration of agglutination between 1:640--10240. It also indicates that among 29 cases in which sonnei bacilli were cultivated and positive HA reaction on sonnei serum was noticed, 93.1% showed high concentration of agglutination (1:640--10240), and that only 6.8%-14.2% had low concentration of agglutina-

Table 3. Sensitivity of "O" Antigens Prepared From Stool Culture to HA Test

Serum	Flexneri Serum							Sonnei Serum						
	1/320	1/640	1/1280	1/2560	1/5120	1/10240	共計	1/320	1/640	1/1280	1/2560	1/5120	1/10240	Total
Cases	9	13	25	17	11	2	77	2	2	3	10	8	4	29
%	11.6	16.8	33.8	22	14.2	2.6	100	6.9	6.9	10.4	34.5	27.5	13.8	100
					88.3%							93.1%		

Table 4. Comparison of Positivity Rates Observed in Bacterial Cultivations and HA Tests Processed During Different Stages of Disease

Stages of Disease	No. of Patients	Positive in Bacterial Exam.		Positive in HA Tests	
		No. of Cases	%	No. of Cases	%
1-3 days	125	67	53.6	85	68.0
4-5 days	43	14	30.2	18	41.8
6-10 days	12	3	25	4	33.3
Longer than 10 days	7	1	12.5	1	12.5

Table 5. Positivity Rates Observed in Three Different Tests on Dysentery Patients in 100 Cases

Diagnosis	No. of Cases	Positive in Bacterial Cultivations			Positive in Rapid Agglutination			Positive in HA Tests		
		Flex.	Son.	%	Flex.	Son.	%	Flex.	Son.	%
Acute Dysentery	100	32	4	36	30	6	36	40	16	56

tion (below 1:160) to both flexneri and sonnei bacilli.

Moreover, the laboratory examination results were analyzed according to different stages of acute dysentery. Table 4 indicates that the highest positive rate observed in bacterial examinations of stool culture and HA tests was noticed during early stages of 1-3 days while patients were suffering the disease.

While bacteria were being cultivated, 100 cases clinically diagnosed as acute dysentery were selected and an HA test along with a rapid agglutination test were processed on those cases to compare sensitivity and specificity of the three methods. The results are tabulated in Table 5.

Table 5 indicates that the positivity rate observed in HA test on antigens prepared from stool culture was 1.5 times as high as that found in bacterial examination and rapid agglutination test on the same kind of culture, although results obtained from any of these three methods were not perfectly in accordance with one another. In 32 cases in which flexneri bacilli were cultivated, 75% (24 cases) showed results from HA test in accordance with one another while in only 50% (16 cases) the results from rapid agglutination test were in accord. In the same number of cases 25% (8 cases) showed results from HA test contradictory to one another, while 50% (12 cases) showed results from rapid agglutination test contradictory to one another. And in the later 32 cases, there were two (16.3%) in which flexneri bacilli were cultivated while sonnei bacille produced in rapid agglutination tests. The rate of contradictions in 311 cases studied in this article was 1.6% which was lower than that in rapid agglutination reactions. While in the four cases in which sonnei bacilli were cultivated, results from HA tests were in accord with one another, in only one of the four cases (25%) the results from rapid agglutination was in

accordance. On the contrary, in another 5 cases in which positive reaction was observed in rapid agglutination tests, negative results were obtained both in bacterial cultivations and HA tests. It is established from analyzing those results that HA reaction is more characteristic than rapid agglutination reaction.

Subsequently, HA test was carried out on 53 specimens of stool culture collected from those who were healthy and who had never suffered dysentery in their life history (Simultaneous bacterial cultivation showed negative reaction.) to illustrate the specificy of this method. Results showed that for concentration of agglutination exceeding 1:160, there were two cases in flexneri serum and three cases in sonnei serum, and that for concentration exceeding 1:320 there were altogether two cases (3.7%). In those two cases one was sonnei serum with agglutination 1:320, the other case showed positive reaction toward flexneri serum with agglutination 1:640. No co-agglutination was noticed on other specimens. In addition, pure cultures were isolated from stool and 107 specimens of coli bacilli isolated were boiled to be "o" antigens which were HA-reacted towards flexneri and sonnei serums. Results showed that only part of the specimens had co-agglutination toward serums with low concentration. Toward sonnei serum with concentration of 1:320 there were six specimens and towards the same serum of 1:640 there was (5.6%). 1 specimen (0.9%) showing reaction: the rest showed no co-agglutination. No co-agglutination was demonstrated towards flexneri with concentration 1:320 by any of the isolated bacilli. But among those 311 cases discussed in this article, 88.3% had positive HA reaction toward flexneri serums with concentration 1:640--1:10240, and 93.1% showed the same reaction toward sonnei serums with concentration 1:640--1:10240. There was a big difference between agglutination concentration of dysentery bacilli and that of coli bacilli. Therefore, it may concluded that although coli bacilli in stool of healthy people show positive reaction to HA test and co-agglutination toward dysentery serums of low concentration, yet the percentage of the bacilli which demonstrate these reactions is in the order of only 3.7-7%. The co-agglutination reaction was found to be caused mostly by serums of concentration at 1:320. And it was also found from experimental results that those showing reaction to a concentration of more than 1:320 will not interfere with the specificity of this method and the interpretation of our experimental results.

Discussion

Efforts to find an early and prompt diagnostic method of detecting dysentery -- a method with characteristic specificity as well as high sensitivity and simplicity of procedure, are significant to both clinical and epidemic studies. Since 1940 a number of reports on methods of precipitating semi-antigens, hemagglutination reaction, and bacteriophage used in diagnostic method have been presented by Russian workers. Many of them have different views of their own concerning characteristic individuality of those methods, especially concerning the method of precipitating semi-antigens. It is well recognized that hemagglutination test is more sensitive than bacterial coagulation reaction in detecting antibodies. But the method in which known immune serums are treated with erythrocytes, infected by "o" antigens that are preapred from boiling stool cultures of patients suffering dysentery in early stages, to undergo HA test has not been reported in literature available to the authors. Learning from the experimental results obtained from 311 cases studied it is concluded that the method mentioned above and used in this experiment results in a positive rate 10% higher than that observed in the bacterial examination and in the rapid agglutination method. In 128 cases in which a positive result was observed in bacterial cultivation, 106 (83%) gave a positive result in HA test also while our method was being employed. In 169 cases in which a negative result was observed in bacterial cultivation, 49 of them (29%) gave a positive result in HA test too. Results varied with the different types of dysentery bacilli: in 96 cases in which flexneri bacilli cultivated, 86% gave positive results in HA test; while in 32 cases in which sonnei bacilli were cultivated, 96.8% gave positive results in HA test. 88.3-93.1% of cases reported in this article showed hemagglutinative concentration in positive HA reaction between 1:640-1:10240. Considering the positive rates shown by the HA test during different stages of the disease, this method had a positive rate 1.3 times as high as that produced in bacterial cultivation during corresponding or simultaneous stages. Therefore, the authors believe that this HA test method is a rather simple method which does not require any special apparatus and has quite high sensitivity and characteristic specificity. This method can be applied to early diagnosis of dysentery of acute type and to such locations as rural and mountainous areas where bacterial cultivation is not possible. These points are advantages of this method. The disadvantages are: (1) When small number of bacilli exist in stool, this

condition may produce a positive result in bacterial cultivation and a negative result in HA test. Among all cases studied in this experiment, 6.8% had positive result in bacterial cultivation and negative result in HA test, thus illustrating this point. (2) Antigens of coli bacilli have resemblances in structure in many cases; therefore, co-agglutination will result in many instances when bacterial cultivation is carried out without selecting bacilli to show any existing contrast.

Concerning the time effect, only 20-30 hours is required to complete all the experimental procedures when this method is used. This is 2.4-3.2 times faster than any other diagnostic method for dysentery ever known. During actual clinical applications the authors also did HA test on erythrocytes infected by antigens prepared from suspension of serums, and results were compared with those obtained from HA test on erythrocytes infected by antigens prepared from precipitate of serums. It was found that there is no prominent difference between results from these two types of HA tests, and that their positive hemagglutinative concentrations have values close to each other, thus conforming to our laboratory results. Since antigens prepared from suspension of serums give equally good results as antigens prepared from precipitate of serums, the procedures of this method can be simplied by suspension antigens being washed three times less than the precipitate, and the disease can be more rapidly detected.

Usually erythrocytes of "o" type of healthy people or those of sheep are used in hemagglutination test by research workers. Since this method is not used to detect special antibodies in serums of patients, erythrocytes of any type of healthy people may be used in this method. Thus this method can be widely used in any area, including those where no sheep is available and where no means exists to examine blood types.

Among all cases on which the authors experimented, 1.9% in which flexneri or sonnei bacilli were cultivated had results from HA test contradictory to those from bacterial cultivation. In addition, a few HA positive cases observed during actual treatment showed co-agglutination toward flexneri or sonnei serums of different concentrations at the same time. This phenomenon can not be satisfactorily explained now and is to be studied.

The question arises if coli communis and coli communior bacilli cause co-agglutination, should this method be used. According to literature available this is the case in reactions of precipitate of semi-antigens, and 16.9% of cases in which bacilli existing in stool of healthy people are cultured produce positive results in the bacterial

cultivation method. But according to results obtained from this experiment, only 3.7% of cases studied showed co-agglutination in HA test toward flexneri or sonnei serums of low concentrations that were mostly below 1:320 with exception of one case (1.8%) of flexneri [illegible] exceeding 1:640; while 106 cases studied in this experiment had a concentration of agglutination in positive HA reaction mostly exceeding 1:1280. And concentration exceeding L;320 (+ + +) is considered a positive result. Therefore, the authors believe that the existence of antigens of coli communis bacilli in stool culture when this method is used, would not interfere with specificty of antigens of dysentery bacilli.

Conclusions

1. This article presents experimental results from 311 cases in which erythrocytes infected by "o" antigens prepared from boiling stool culture were HA-tested towards dysentery serums of known type while dysentery bacilli were being cultivated. In 100 cases out of those 311, rapid agglutination test was also processed for comparison. It was found that the positivity rate of HA tests was 10% higher than that of bacterial examinations and rapid coagulation tests.

2. HA test on antigens prepared from boiling stool culture has rather high sensitivity and specificity in early diagnosis of dysentery, and types of dysentery bacilli can also be determined. Concentration of agglutination has to exceed 1:320 to be considered a positive result. And the existence of antigens of coli communis bacilli in stool culture would not interfere with the specificity of "o" antigens of dysentery bacilli.

3. This method recommended by the authors is easily employed. It does not require special apparatus and culture media involving re-infection; and the result can be reported 20-30 hours after starting the test. This method can be used in general clinical laboratories, thus opening a new field of activities by them and suggesting the possibility of developing new methods of diagnosis of dysentery as a serious disease. But this method can not entirely replace the technique of bacterial cultivation.

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5. Tests on Resistance of Local Strains of Dysentery Bacilli to Common Drugs

/Following is a translation of excerpts from an article written by Yeh Yu-lin of Microbiology Laboratory, Health and Quarantine Station, Hupeh Province, and by T'ang Jen-fang of Academy for Health Workers, Hupeh Province, appearing in Wei-sheng-wu Hsueh-pao (ACTA Microbiologica Sinica), Vol. 7, No. 1-2, May 1959, pp. 128-131./

It was in September 1956 that we -- workers from the Health and Quarantine Station of Hupeh Province -- were working on vaccination and clinical treatment of dysentery at the construction base in Chien-li Hsien, Hupeh Province. We noticed that in a number of patients the disease changed from acute to chronic type, and that sulfaguanidine could not do anything effective for some of these patients. This led us to believe that local strains of dysentery bacilli might have resistance to certain drugs. And the inhibitory effect of drugs, commonly used to treat dysentery on clinically isolated dysentery bacilli was tested in this experiment conducted by the authors.

Bacterial Specimens and Reagents

1. Strain of dysentery bacilli: 104 specimens of dysentery bacilli isolated at this locale plus 20 specimens of standard strain which originated from Russia and Czechoslovakia and were offered by the Bureau of Biological Products, Wu-han.
2. Reagents:
   1) 20% sulfathiazole solution: Manufactured by the State-operated Wuhan Drug Co., Wuhan. Solution was in 10 portions of 2 ml each. After preparation, the total weight of the solution was 4 gm.
   2) Sulfaguanidine: Manufactured by China Drug Company.
   3) Syntomycin: Made by the State-operated Wuhan Drug Co., Wuhan.

1 gm of each of these reagents was weighed out, placed in 100 ml of medium of coagulated peptone solution, and heated to effect solution. The solution was diluted to different concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml and 0.3125 mg/ml. More dilute concentrations were made on syntomycin solution to 80 g/ml, 40 g/ml, 20 g/ml, 10 g/ml, 5 g/ml, and 2.5 g/ml.

4) Coptis root: The so-called Ssu-ch'uan coptis root was purchased in the Chen-tai-yi Chinese Drug Store in Han-kow. 30 gm of coptis root and 100 ml of water were placed in a flask and heated to boiling on an alcohol burner for two hours. The boiling solution was filtered through filter paper and concentrated to a volume of 30 ml.

30 ml of concentrated coptis root solution was added to 300 ml of culture medium to make 1:10 concentration. Further dilutions of 5%, 2.5%, 1.25%, 0.625%, 0.3125% and 0.15625% were made subsequently.

Experimental Procedures

1. The culture solutions containing differing amounts of reagents were distributed in test tubes with 2 ml of solutions in each tube. It was sterilized in a steam, high-pressure sterilizer with 5-lb pressure for 30 min. The tubes were then lined up according to different concentrations with six tubes in each row.

2. Meat extract in which dysentery bacilli had been cultivated for 18 hours was inoculated with a platinum loop to media consisting of solutions in which different amounts of reagents were dissolved. The media were warmed in an incubator at 37° C. for 18 hours and observations were made. Those strains of bacteria which had been cultured in media but did not grow were considered sensitive to the drugs; those that did grow were considered resistant to those drugs.

Experimental Results

The sources of clinically isolated bacterial strains:

	No. of patients with positive condition	%	Shigella Flexneri	Shigella boyd	Schmitz's bacillus
The sick 355	96	27.0	84	6	6
The healthy 56	8	14.2	5	2	1

Discussion and Conclusions

It was learned from the experimental results that sulfaguanidine of different concentrations in test tubes

has no inhibotory effect on dysentery bacilli and that all bacterial strains have some resistance to common drugs. The inhibitory effect of sulfathiazole on the same bacilli was found to be greater than that of sulfaguanidine, but it was also found that it induces resistance to a certain extent.

The bacterial growth in test tubes is not necessarily similar to that in the human body. However, the experimental results which indicated that 95% of all the bacterial strains tested has resistance to drugs of as high a concentration as 10 mg/ml justify consideration of the continued use of sulfaguanidine to treat dysentery in that location.

Syntomycin was found to have a powerful inhibitory effect on bacteria cultivated in test tubes. An absolute inhibitory effect of syntomycin was noted when its solution has a concentration of 10 g/ml, and no bacterial strain was found to have any resistance to this drug.

Coptis root of high concentration was noted to have an inhibitory effect on growth of dysentery bacilli. It was also observed that the growth of local strains of dysentery bacilli in coptis root solution is similar to that of standard strains in the same solution. Therefore, the authors believes that dysentery bacilli have not yet developed any resistance to coptis root.

As a conclusion, the authors would like to call the attention of research workers studying epidemic diseases to the fact that certain strains of dysentery bacilli have developed resistance to some of the common drugs.

Author's Statement

The authors studied 104 strains of dysentery bacilli isolated in Hupeh for their sensitivity to sulfaguanidine, sulfathiazole, syntomycin, and to coptis root. The results obtained agreed well with those reported elsewhere, showing a high percentage resistant to the sulfa drugs, while only a few strains were resistant to syntomycin or to coptis root.

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